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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/918,702 | 07/31/2001 | Nissim Benvenisty | 1822/113 | 3581 |

2101 7590 06/01/2004

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| EXAMINER |
|---|
| CROUCH, DEBORAH |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1632 | |

DATE MAILED: 06/01/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/918,702 | BENVENISTY, NISSIM | |
| | Examiner | Art Unit | |
| | Deborah Crouch, Ph.D. | 1632 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *17 February 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *8-16 and 48-69* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *8-16 and 48-69* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *7/31/01* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on February 17, 2004 has been entered. Claims 1-16 and 18-69 are pending.

Applicant states in the remarks that claims 1-7 and 17-47 are canceled. However, applicant should have put this request under the claims section of the response. In response to this office action, should applicant still want to cancel claims 1-7 and 17-47, the request should be put in a separate place "amendments to the claims."

Newly submitted claims 49, 50, 53, 54, 56-59, 61-64 and 66-69 are directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: The newly added claims are directed to methods of directed differentiation of specific cell types not present in the originally filed claims. As such, claims 49, 50, 53-59, 61-64 and 66-69 require search and consideration not required for the originally present claims 8-16. These claims are drawn to materially different methods of inducing human ES cell differentiation into patentably distinct cells types, which would require materially different separate protocols. No one method is required for the implementation of any of the other methods claimed in claims 49, 50, 53, 54, 56-59, 61-64 and 66-69.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 49, 50, 53, 55, 56-59, 61-64 and 66-69 are

withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

Applicant is reminded that an RCE is not a new case, but is continued prosecution on the originally claimed or elected invention (MPEP 706.7(h) see Table, items 4 and 10). In the present situation, originally presented claim 12 encompassed generically differentiation into blood cells, which are of mesodermal origin and originally presented claims 13-16 encompassed neurons, which are of ectodermal organ. Claims 8-11 are linking claims. Thus, as these specific cell types have been de facto examined by the examiner, generic and species claims 48, 51, 52, 55, 60 and 65 filed February 17, 2004 with the request for continued prosecution have been included in the present examination.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 10 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for culturing embryonic cells on an extracellular matrix, does not reasonably provide enablement for culturing embryonic cells without an extracellular matrix. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claim 12 does not require the presence of an extracellular matrix for the monolayer of embryonic cells. The human embryonic cells derived from EB's require the presence of an extracellular matrix or feeder cells to remain undifferentiated. Thomson teaches that human ES cells differentiated in culture in the absence of feeder cells; that is when plated directly (page 1146, col. 1, parag. 2, lines 12-17). The specification teaches that dissociated EB

cells were plated on tissue culture plates coated with fibronectin, an extracellular matrix (specification, page 14, lines 20-21). It is unclear that embryonic cells grown on feeder cells, if applicant has support for such a limitation, would be considered a monolayer.

Thus, at the time of the instant invention, the skilled artisan would have needed to engage in an undue amount of experimentation to make and use the invention as claimed without a predictable degree of success.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 8, 9 11, 12, 60 and 65 are rejected under 35 U.S.C. 103(a) as being unpatentable over in Thomson et al (1998) Science 282, 1145-1147 in view of Shamblott et (1998) Proc. Natl. Acad. Sci. (USA) 95, 13726-13731 and further in view further in view of Yuen et al. (1998) Blood 91, 3202-3209.

Thomson teaches human ES cells that retain the ability to form all three embryonic germ layers (page 1146, col. 1, parag. 2, lines 1-3).

Shamblott teaches the production of human EB's from human primordial germ cells (PGC's), cultured with or without LIF (page 13729, col. 1-2, bridg. parag). Primordial germ cells (PGC's) are the source for embryonic germ (EG) cells, and in mice is a cell that shares pluripotent characteristics with mouse ES cells (page 13726, col. 1, lines 1-5). Shamblott also teaches that human EG cells have several characteristics in common with mouse ES and EG cells, especially the ability to develop into all three germ layers (page 13726. col. 2, parag. 1 and page 13729, col. 2, parag. 5, lines 1-11).

Yuen teaches the production of embryoid bodies (EB's) in suspension from mouse ES cells (page 3203, col. 1, lines 3-7). After aggregation, the EB's were dissociated with trypsin to produce embryonic cells which were then cultured on an extracellular matrix, methylcellulose (page 3203, col. 1, parag. 1, lines 1-3). The embryonic cells/methylcellulose cultures were grown in the presence of transferrin, bFGF and Epo, and resulting primitive erythroid cells were culture in media containing several interleukins, Il-3, -11 and -6, all of which are growth factors directing differentiation to hematopoietic stem cells (page 3203, col.1, parag. 1, lines 4-10 and parag. 2, lines 1-5). Erythroid cells, a type of blood cells, arise from the mesoderm.

Therefore at the time of the present invention, it would have been obvious to the ordinary artisan to produce human EB's from human ES cells as taught by Thomson using the methodology of Shamblott, and then, following the methodology of Yuen for mouse EB's, produce a culture of embryonic cells by dissociating human EB's, and culture the embryonic cells in the presence of transferrin, bFGF, and EPO and IL3 to observe the formation of primitive erythroid cells. Motivation can be found in Thomson teaching that human ES cells will offer insights into developmental events that cannot be studied in a human embryo but which have important consequences in birth defects, infertility and pregnancy loss (page 1146, col. 1, parag. 3, lines 1-6). Thus, at the time of filing, the ordinary artisan would have had sufficient teaching, suggestion and motivation to reach the claimed invention to develop an in vitro method studying human erythrocyte differentiation from ES cells.

Applicant should be aware that the phrase in claim 8, "for differentiating in the presence of at least one exogenous factor for an effective period of time" is regarded as an intended use for the cells, and not, necessarily, part of the presently claimed invention. There is no limitation in the claims that the embryonic cells produced by dissociating

embryoid bodies are themselves, specifically, cultured in the presence of an exogenous factor. Thus Yuen teaching the culture of EB's with exogenous factors and then observing the formation of erythroid cells reads on the claimed invention.

Claims 8-12, 14-16, 48, 51 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al (1998) Science 282, 1145-1147 in view of Shamblott et (1998) Proc. Natl. Acad. Sci (USA) 95, 13726-13731 and further in view of Bain et al (1995) Devel. Biol. 168, 342-357.

Thomson teaches human ES cells that retain the ability to form all three embryonic germ layers (page 1146, col. 1, parag. 2, lines 1-3).

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Bain teaches the production of EB's in suspension by the aggregation of mouse ES cells (page 343, col.2, parag. 2, lines 23-26). EB's were cultured in median comprising retinoic acid (RA) (page). EB's were then dissociated with trypsin and plated on laminin-coated dishes (page 344, col. 1, lines 3-6). The monolayer cells, plated on laminin coated plates, developed into neuron-like cells having neuritic processes (page 347, col. 1, lines 3-4). Neurons, like other brain cells are of ectodermal origin.

Therefore at the time of the present invention, it would have been obvious to the ordinary artisan to produce human EB's from human ES cells as taught by Thomson using the methodology of Shamblott, and then following the methodology of Bain for mouse EB's,

culture the human EB's in the presence of retinoic acid, subsequently dissociate the human EB's and plate the embryonic cells thereby produced on a laminin coated plate to produce a monolayer of embryonic cells to observe neuron-like cells with neuritic processes. Motivation can be found in Thomson teaching that human ES cells will offer insights into developmental events that cannot be studied in a human embryo but which have important consequences in birth defects, infertility and pregnancy loss (page 1146, col. 1, parag. 3, lines 1-6). Thus, at the time of filing, the ordinary artisan would have had sufficient teaching, suggestion and motivation to reach the claimed invention to develop an in vitro system to observe neuronal cells differentiation from human ES cells.

Claims 8, 11, 13, 48, 51 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al (1998) Science 282, 1145-1147 in view of Shamblott et (1998) Proc. Natl. Acad. Sci (USA) 95, 13726-13731 and further in view of Bain et al (1995) Devel. Biol. 168, 342-357 and Wobus et al (1988) Biomed. Biochim. Acta 47, 965-973.

Thomson teaches human ES cells that retain the ability to form all three embryonic germ layers (page 1146, col. 1, parag. 2, lines 1-3).

Shamblott teaches the production of human EB's from human primordial germ cells (PGC's), cultured with or without LIF (page 13729, col. 1-2, bridg. parag). Primordial germ cells (PGC's) are the source for embryonic germ (EG) cells, and in mice is a cell that shares pluripotent characteristics with mouse ES cells (page 13726, col. 1, lines 1-5). Shamblott also teaches that human EG cells have several characteristics in common with mouse ES and EG cells, especially the ability to develop into all three germ layers (page 13726. col. 2, parag. 1 and page 13729, col. 2, parag. 5, lines 1-11).

Bain teaches the production of EB's in suspension by the aggregation of mouse ES cells (page 343, col.2, parag. 2, lines 23-26). EB's were cultured in median comprising retinoic acid (RA) (page). EB's were then dissociated with trypsin and plated on laminin-

coated dishes (page 344, col. 1, lines 3-6). The monolayer cells, plated on laminin coated plates, developed into neuron-like cells having neuritic processes (page 347, col. 1, lines 3-4).

Wobus teaches the production of neuron-like cells in culture when mouse EB's are cultured in the presence of nerve growth factor (NGF) (page 968, line 9 to page 969, line 2). Wobus states that NGF is important for the survival of neurons during embryonic development, for growth of sensory and sympathetic ganglia and for differentiation and maintenance of specific neuronal function (page 965, parag. 2, lines 5-8).

Therefore at the time of the present invention, it would have been obvious to the ordinary artisan to produce human EB's from human ES cells as taught by Thomson using the methodology of Shamblott, and then following the methodology of Bain for mouse EB's, culture the human EB's in the presence of NGF as taught by Wobus, subsequently dissociate the human EB's and plate the embryonic cells thereby produced on a laminin coated plate to produce a monolayer of embryonic cells to observe neuron-like cells with neuritic processes. Motivation can be found in Thomson teaching that human ES cells will offer insights into developmental events that cannot be studied in a human embryo but which have important consequences in birth defects, infertility and pregnancy loss (page 1146, col. 1, parag. 3, lines 1-6). Additional motivation can be found in Wobus stating the results disclosed establish an experimental cell model to study the effect on differentiation of stem cells (page 970, parag. 2). Thus, at the time of filing, the ordinary artisan would have had sufficient teaching, suggestion and motivation to reach the claimed invention to develop an in vitro system to study the role of NGF on human neuronal cell differentiation from ES cells.

Claim 55 is rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson et al (1998) Science 282, 1145-1147 in view of Shamblott et (1998) Proc. Natl. Acad. Sci (USA) 95, 13726-13731 and further in view of Bain et al (1995) Devel. Biol. 168, 342-357 and Wobus et al (1988) Biomed. Biochim. Acta 47, 965-973.

Thomson teaches human ES cells that retain the ability to form all three embryonic germ layers (page 1146, col. 1, parag. 2, lines 1-3).

Shamblott teaches the production of human EB's from human primordial germ cells (PGC's), cultured with or without LIF (page 13729, col. 1-2, bridg. parag). Primordial germ cells (PGC's) are the source for embryonic germ (EG) cells, and in mice is a cell that shares pluripotent characteristics with mouse ES cells (page 13726, col. 1, lines 1-5). Shamblott also teaches that human EG cells have several characteristics in common with mouse ES and EG cells, especially the ability to develop into all three germ layers (page 13726. col. 2, parag. 1 and page 13729, col. 2, parag. 5, lines 1-11).

Bain teaches the production of EB's in suspension by the aggregation of mouse ES cells (page 343, col.2, parag. 2, lines 23-26). EB's were cultured in median comprising retinoic acid (RA) (page). EB's were then dissociated with trypsin and plated on laminin-coated dishes (page 344, col. 1, lines 3-6). The monolayer cells, plated on laminin coated plates, developed into neuron-like cells having neuritic processes (page 347, col. 1, lines 3-4).

Wobus teaches the production of endodermal cells when mouse ES cells are cultured in the presence of nerve growth factor (NGF) and permitted to form EB's (page 969, lines 2-4).

Therefore at the time of the present invention, it would have been obvious to the ordinary artisan to produce human EB's from human ES cells as taught by Thomson using the methodology of Shamblott, and then following the methodology of Bain for mouse EB's,

culture the human EB's in the presence of NGF as taught by Wobus, subsequently dissociate the human EB's and plate the embryonic cells thereby produced on a laminin coated plate to produce a monolayer of embryonic cells to observe neuron-like cells with neuritic processes. Motivation can be found in Thomson teaching that human ES cells will offer insights into developmental events that cannot be studied in a human embryo but which have important consequences in birth defects, infertility and pregnancy loss (page 1146, col. 1, parag. 3, lines 1-6). Additional motivation can be found in Wobus stating the results disclosed establish an experimental cell model to study the effect on differentiation of stem cells (page 970, parag. 2). Thus, at the time of filing, the ordinary artisan would have had sufficient teaching, suggestion and motivation to reach the claimed invention to develop an in vitro system to study the role of NGF on human endodermal cell differentiation from ES cells.

Applicant argues that Reubinoff teaches that techniques used to produce EB's from mouse cells would not produce EB's from human ES cells. Applicant argues that Reubinoff teaches human ES cells did not for EB's using the hanging drop method or as aggregates on bacterial Petri dishes. Applicant argues that Reubinoff states that considerable cell death occurred using these methods. Applicant argues that when human ES cells were cultivated to high density on a feeder layer, there was no consistent pattern of structural organization suggestive of the formation of embryoid bodies as is formed in EB's from mouse ES cells. Applicant argues that these results are consistent with results obtained when EB formation was attempted using marmoset and rhesus monkey ES cells. These arguments are not persuasive.

Reubinoff states that when human ES cells were cultivated to a high density that organized EB's failed to form. Likewise, Reubinoff states that the cultivation of clumps of ES cells by either the hanging drop or as aggregates on bacterial plates resulted in cell death.

However, there is no discussion as to the EB outcome when lower density cultures were a source of cells. There is also no discussion as to the EB outcome using smaller clumps of cells or single cells. Shamblott offers methods, albeit using human primordial germ cells or their derivatives, to produce EB's that are described as having an organized structure (Shamblott, page 13730, fig. 5). Reubinoff does not provide a cellular concentration that defines "high density." All Reubinoff states is the human ES cells were cultured at a high density for 4-7 weeks, and these cells did not form EB's with structure or the cells died. These teachings do not address a case where less dense cell concentrations or where the cells were not cultured so long. Some experimentation is permitted in 103 especially when a positive teaching is provided as Shamblott. It is noted that applicant's specification does not teach the concentration of human ES cells plated to produce EB's (specification, page 14, lines 17-19). No other guidance to cell plating number could be found. Also, the results of Shamblott obviate any difficulties seen with other primate ES cells.

Applicant argues that human ES cells form embryoid bodies with or without LIF and that this difference would motivate one to develop new protocols from those used to produce mouse EB's, which develop EB's only in the absence of LIF. This argument is not persuasive.

It is not seen that producing human EB's, that is culture with or without LIF affect a differentiation protocol. The presence or absence of LIF is not in the claims. Further as applicant argued, this phenomenon was known in the art at the time of filing. Both Thomson and Shamblott currently cited references acknowledge this effect. Differentiation as claimed is concerned with the embryonic cells isolated from the EB's. As long as you have EB's, the formation, the mouse differentiation protocols would be applicable. Furthermore, the cited references in combination meet the limitations in the claims.

Arguments to Keller are not addressed, as Keller is no longer cited in the rejection.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Deborah Crouch, Ph.D. whose telephone number is 571-272-0727. The examiner can normally be reached on M-Th, 8:30 AM to 7:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on 571-272-0408. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Deborah Crouch, Ph.D.
Primary Examiner
Art Unit 1632

May 27, 2004